AR201-13793B

HUMAN HEALTH ENDPOINTS (NON SIDS)
OESTROGENIC ACTIVITY

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NX 4708 (distilled cashew nut shell liquid)
Source: Cardanol Chemicals N.V., Lot No. LT-0481

METHOD

- **Method:** Routledge and Sumpter (1996)
- **Test Type:** Recombinant Yeast Screen A ssay
- **System of testing**: Non bacterial
- **GLP:** Yes
- **Year:** 1999
- **Species/Strain:** Saccharomyces cerevisiae, recombinant strain containing the human oestrogen receptor (hER) and the reporter gene *lac -Z* (encoding for the enzyme ß-galactosidase).
- **Concentrations tested:** 0.049, 0.098, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 mg/l
- **Statistical Methods:** None

Remarks:

- **Test Design:**
  - **Number of replicates:** 2
  - **Frequency of dosing:** Single
  - **Positive control:** 17β-estradiol, Bisphenol A
- **Solvent:** Ethanol

RESULTS

- **Result:** Negative

Remarks: None

CONCLUSIONS

Remarks:

No oestrogenic activity was observed at all concentrations tested.

In accordance with current regulatory guidelines for the environmental classification of chemicals it was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/l.

Care should be taken in the interpretation of these results, as a negative result in this in vivo study does not necessarily indicate that the material will not have an oestrogenic effect in the environment.

Bisphenol A was determined to be 3500 times less potent that 17β-estradiol.

The response of the recombinant yeast screen to both of the positive control materials was comparable to published results thereby confirming the suitability of the innoculum and culture conditions.

REFERENCES (Free Text)

SafePharm Laboratories Ltd., Cardolite NX4708: Assessment of oestrogenic activity using a recombinant yeast screen assay, Report No. 814/005, June 1999

Routledge EJ and Sumpter JP, 1996, Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen, Environmental Toxicology and Chemistry 15: 241-248

OTHER

- **Last Changed:** 20 May 2002
- **Order number for sorting:** 3

HUMAN HEALTH ENDPOINTS (NON SIDS)
OESTROGENIC ACTIVITY

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 700 (distilled cashew nut shell liquid)
Source: Cardanol Chemicals N.V., Lot No. GT457

METHOD

- **Method:** Routledge and Sumpter (1996)
- **Test Type:** Recombinant Yeast Screen Assay
- **System of testing**: Non bacterial
- **GLP:** Yes
- **Year:** 1999
- **Species/Strain:** Saccharomyces cerevisiae, recombinant strain containing the human oestrogen receptor (hER) and the reporter gene *lac-Z* (encoding for the enzyme ß-galactosidase).
- **Concentrations tested:** 0.049, 0.098, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 mg/l
- **Statistical Methods:** None

Remarks:

- **Test Design:**

  - **Number of replicates:** 2
  - **Frequency of dosing:** Single
  - **Positive control:** 17β-estradiol, Bisphenol A

- **Solvent:** Ethanol

RESULTS

- **Result:** Negative

Remarks: None

CONCLUSIONS

Remarks:

No oestrogenic activity was observed at all concentrations tested.

In accordance with current regulatory guidelines for the environmental classification of chemicals it was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/l.

Care should be taken in the interpretation of these results, as a negative result in this in vivo study does not necessarily indicate that the material will not have an oestrogenic effect in the environment.

Bisphenol A was determined to be 3500 times less potent that 17β-estradiol.

The response of the recombinant yeast screen to both of the positive control materials was comparable to published results thereby confirming the suitability of the innoculum and culture conditions.

REFERENCES (Free Text)

SafePharm Laboratories Ltd., Cardolite NC700: Assessment of oestrogenic activity using a recombinant yeast screen assay, Report No. 814/004, June 1999

Routledge EJ and Sumpter JP, 1996, Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen, Environmental Toxicology and Chemistry 15: 241-248

OTHER

- **Last Changed:** 15 May 2002
- **Order number for sorting:** 2

HUMAN HEALTH ENDPOINTS (NON SIDS)
SKIN SENSITIZATION

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 700 (distilled cashew nut shell liquid)
Source: Cardolite Corporation, Lot No.: EQ-1

METHOD

- **Method:** OECD 406, 'Skin Sensitisation'.
- **Species/strain:** Albino Dunkin Hartley guinea pigs.
- **Concentration**
  - **Intradermal induction:** 1% w/v in liquid paraffin
    1% w/v in a mixture of Freund's Complete Adjuvant plus distilled water (1:1)
  - **Topical induction:** 25% v/v in liquid paraffin
  - **Topical challenge:** 5% and 2% v/v in liquid paraffin
- **No of animals/sex/dose:** 20 females/dose
- **Vehicle:** Liquid Paraffin BP
- **GLP:** Yes
- **Year:** 1996

Remarks: None

RESULTS

- Sensitization rate: 14/20 (70%) sensitised
- Result: Positive

Remarks:

Skin reactions observed after intradermal induction: Well-defined erythema (grade 2) was commonly noted at the intradermal injection sites at the 24-hour observation. Incidents of moderate to severe erythema were also noted at this time. Well-defined erythema persisted at all intradermal injection sites at the 48-hour observation.

Skin reactions observed after topical induction: Very slight or well-defined erythema (grades 1 or 2) with or without very slight oedema (grade 1), was commonly noted at the topical induction sites at the 1-hour observation. Incidents of fissuring of the skin, or bleeding were also noted at this time. The bleeding was probably caused by self-inflicted scratching of the skin.

Skin reactions observed after topical challenge with 5% v/v Cardolite NG700: Very slight or well-defined erythema (grade 1 or 2) was noted at the challenge sites of eleven animals at the 24-hour observation. Very slight oedema (grade 1) was also noted at five of these sites at this observation. Very slight erythema (grade 1) was noted at the challenge sites of 14 animals at the 48-hour observation, with very slight oedema (grade 1) at two of these sites.

Desquamation was seen at the challenge sites of seven animals. No evidence of erythema or oedema was seen at the 72-hour observation, although the presence of desquamation precluded evaluation of erythema at the challenge sites of none animals at this time.

Skin reactions observed after topical challenge with 2% v/v Cardolite NC700: Very slight or well-defined erythema (grade 1 or 2) was noted at the challenge sited of six animals at the 24-hour observation. Very slight oedema (grade 1) was also noted at one of these sites at this observation. Very slight erythema (grade 1) was noted at the challenge sited of five animals at the 48-hour observation. No skin reactions were noted at the challenge sites of two of these animals at the 24-hour observation. Desquamation was noted at one challenge site at the 48-hour observation. Very slight erythema (grade 1) persisted at the challenge site of one animal at the 72-hour observation. Desquamation was noted at the challenge sites of three animals at this time.

Clinical observations: All animals showed an expected gain in bodyweight over the study period. No signs of ill-health were noted in any animal.

CONCLUSIONS

Remarks: Cardolite NC-700 produced a 70% (14/20) sensitisation rate in this study and was classified as a strong as a strong sensitiser.

REFERENCES (Free Text)

SafePharm Laboratories Ltd., Determination of the skin sensitisation potential of Cardolite NC-700 and assessment of cross-sensitisation potential with poison ivy oil, Cardolite NC-513, Cardolite NC-514, Cardolite NC-541 and Cardolite NC0558, Report No. 661/010, February 1996

OTHER

- Last Changed: 20 May 2002
- Order number for sorting: 1

Remarks: None

ECOTOXICITY ENDPOINTS
11. TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

TEST SUBSTANCE

- **Identity:** Cashew Nut Shell Liquid

Remarks: Test substance: Cardanol (CAS No. 37330-39-5), Cardol (CAS No. 57486-25-6)

METHOD

- **Method:** Calculation using ECOSAR v.0.99e
- **Type:** N/A
- **GLP:** No
- **Year:** 2002
- **Species:** Algae

Remarks: None

RESULTS

- **Unit:** mg/L
- **EC_{50} at 96 hours:** 0.00011 – 0.00034 (Cardanol)
  0.00031 – 0.00096 (Cardol)

Remarks: The predicted EC_{50} values vary with the degree of unsaturation in the alkyl side chain of Cardanol and Cardol as follows:

	EC_{50}, mg/L			
	unsaturated	monoene	diene	triene
Cardanol	0.00011	0.00017	0.00026	0.00034
Cardol	0.00031	0.00048	0.00072	0.00096

CONCLUSIONS

Remarks:

Estimation using ECOSAR v0.99e predict Cardanol and Cardol, the two major components of distilled and technical grade Cashew Nut Shell Liquid, to be toxic to algae.

DATA QUALITY

Reliabilities: 4, Not Assignable

Remarks:

Estimation using ECOSAR v.0.99e

REFERENCES (Free Text)

ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

OTHER

- Last Changed: 24 April 2002
- Order number for sorting: 1

Remarks:

HUMAN HEALTH ENDPOINTS
15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)
Source: Cardolite Corporation, Lot No.: LP-2

METHOD

- **Method:** OECD 473
- **Test Type:** Chromosomal aberration test
- **System of testing:** Non bacterial
- **GLP:** Yes
- **Year:** 1995
- **Species/Strain:** Human Lymphocytes
- **Metabolic activation:** S9-mix, Rat liver cells, Aroclor induced, 1 ml
- **Concentrations tested:** Expt. 1 (20h harvest): 0, 6.25, 12.5, 25 µg/ml (-S9)
  0, 3.125, 6.25, 12.5 µg/ml (+S9)
  Expt. 2 (20h harvest): 12.5, 25, 37.5 µg/ml (-S9)
  0.78, 1.56, 3.125, µg/ml (+S9)
  Expt. 2 (44h harvest): 25 µg/ml (-S9)
  3.125 µg/ml (+S9)

- **Statistical Methods:** Fisher's Exact test or Chi-squared test

Remarks:

- **Test Design**

  - **Number of replicates:** 2
  - **Positive control:** Ethyl methanesulphonate (EMS) (-S9), cyclophosphamide (+S9)
  - **Negative control:** Solvent vehicle

- **Solvent:** Dimethylsulfoxide

RESULTS

- **Result:** Negative
- **Cytotoxic concentration**

  - **With metabolic activation:** 12.5 µg/ml
  - **Without metabolic activation:** >25 µg/ml

- **Genotoxic effects**
  - **With metabolic activation:** None
  - **Without metabolic activation:** None

- **Statistical results:** The test material did not induce a significant increase in the frequency of cells with chromosome aberrations or polyploid cells in either the presence or absence of a liver enzyme metabolizing system.

Experiment 1: Harvest Time 20 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	1	0	0	0	1	1	1	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	0	0	1	0	0	0	1	1	1	1
			(0.0)	(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
6.25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)
12.5 µg/ml	A	100	2	1	0	0	0	0	3	1	3	1
	B	100	0	0	0	1	0	0	1	1	1	1
	Total	200	2	1	0	1	0	0	4	2	4	2
			(1.0)	(0.5)	(0.0)	(0.5)	(0.0)	(0.0)	(2.0)	(1.0)	(2.0)	(1.0)
25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	1	1	0	0	0	0	2	1	2	1
	Total	200	2	1	0	0	0	0	3	1	3	1
			(1.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(0.5)	(1.5)	(0.5)
Positive Control EMS 500 µg/ml	A	50	31	14	7	2	1	0	55	24	33	21
	B	50	13	18	8	2	0	0	41	28	29	24
	Total	100	44	32	15	4	1	0	96	52	62***	45***
			(44.0)	(32.0)	(15.0)	(4.0)	(1.0)	(0.0)	(96.0)	(52.0)	(62.0)	(45.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells *** represents $p \leq 0.001$

Experiment 1: Harvest Time 20 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	0	1	0	0	0	0	1	1	1	1
	Total	200	0	1	0	0	0	0	1	1	1	1
			(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
1.56 µg/ml	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	0	1	0	0	0	0	1	1	1	1
	Total	200	0	1	0	0	0	0	1	1	1	1
			(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
3.125 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	1	0	0	0	0	0	1	0	1	0
	Total	200	2	0	0	0	0	0	2	0	2	0

			(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.0)
6.25 µg/ml	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	4	0	0	0	0	0	4	0	4	0
	Total	200	4	0	0	0	0	0	4	0	4	0
			(2.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(2.0)	(0.0)	(2.0)	(0.0)
Positive Control CP 25 µg/ml	A	100	4	0	0	1	0	0	5	1	5	1
	B	100	1	2	0	2	0	0	5	4	4	3
	Total	200	5	2	0	3	0	0	10	5	9**	4
			(2.5)	(1.0)	(0.0)	(1.5)	(0.0)	(0.0)	(5.0)	(2.5)	(4.5)	(2.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells

** represents $p \leq 0.01$

Experiment 2: Harvest Time 20 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	2	0	0	0	0	0	2	0	2	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	2	0	0	0	0	0	2	0	2	0
			(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.0)
12.5 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)
25 µg/ml	A	100	1	1	0	0	0	0	2	1	2	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	1	0	0	0	0	2	1	2	1
			(0.5)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)
37.5 µg/ml	A	100	1	1	0	0	0	0	2	1	2	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	1	0	0	0	0	2	1	2	1
			(0.5)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)
Positive Control EMS 500 µg/ml	A	100	6	9	4	0	0	0	19	13	13	11
	B	100	16	17	2	1	0	0	36	20	26	15
	Total	200	22	26	6	1	0	0	55	33	39***	26***
			(11.0)	(13.0)	(3.0)	(0.5)	(0.0)	(0.0)	(27.5)	(16.5)	(19.5)	(13.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells

*** represents $p \leq 0.001$

Experiment 2: Harvest Time 20 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	1	1	1	0	0	0	3	2	3	2
	B	100	0	1	0	0	0	0	1	1	1	1

	Total	200	1	2	1	0	0	0	4	3	4	3
			(0.5)	(1.0)	(0.5)	(0.0)	(0.0)	(0.0)	(2.0)	(1.5)	(2.0)	(1.5)
0.78	A	100	0	0	0	0	0	0	0	0	0	0
µg/ml	B	100	0	3	0	0	0	0	3	3	3	3
	Total	200	0	3	0	0	0	0	3	3	3	3
			(0.0)	(1.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)
1.56	A	100	1	0	0	0	0	0	1	0	1	0
µg/ml	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)
3.125	A	100	0	0	0	0	0	0	0	0	0	0
µg/ml	B	100	1	0	0	0	1	0	2	1	2	1
	Total	200	1	0	0	0	1	0	2	1	2	1
			(0.5)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)
Positive	A	100	5	4	0	1	0	0	10	5	9	5
Control	B	100	6	0	2	1	0	0	9	3	8	3
CP	Total	200	11	4	2	2	0	0	19	8	17**	8
25			(5.5)	(2.0)	(1.0)	(1.0)	(0.0)	(0.0)	(9.5)	(4.0)	(8.5)	(4.0)
µg/ml												

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells ** represents $p \leq 0.01$

Experiment 2: Harvest Time 44 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	0	3	0	0	0	0	3	3	3	3
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	0	3	0	0	0	0	3	3	3	3
			(0.0)	(1.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)
25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells

Experiment 2: Harvest Time 44 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	0	1	0	0	1	1	1	1
	B	100	0	1	0	1	0	0	2	2	2	2
	Total	200	0	1	0	2	0	0	3	3	3	3
			(0.0)	(0.5)	(0.0)	(1.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)
25 µg/ml	A	100	2	1	0	1	0	0	4	2	4	2
	B	100	1	0	0	0	0	0	1	0	1	0
	Total	200	3	1	0	1	0	0	5	2	5	2

			(1.5)	(0.5)	(0.0)	(0.5)	(0.0)	(0.0)	(2.5)	(1.0)	(2.5)	(1.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells

Experiment 1: Mean Frequency of Polyploid Cells (%)

Dose Level µg/ml	20 Hours	
	Without S9	With S9
0	0.0	0.0
1.56	-	0.5
3.125	-	0.0
6.25	0.0	0.0
12.5	0.0	-
25	0.0	-
EMS 500	0.0	-
CP 25	-	0.0

Experiment 2: Mean Frequency of Polyploid Cells (%)

Dose Level µg/ml	Without S9		Dose Level µg/ml	With S9	
	20 hours	44 hours		20 hours	44 hours
0	0.0	0.5	0	0.0	1.0
12.5	0.5	-	0.78	0.0	-
25	0.0	0.5	1.56	1.0	-
37.5	0.0	-	3.125	1.0	0.0
EMS 500	0.0	-	CP 25	0.5	-

Remarks:

Experiment 1: Mitotic Index (20-hour harvest)

Dose Level µg/ml	Without S9				With S9			
	A	B	Mean	% of Control	A	B	Mean	% of Control
0	5.80	6.25	6.03	100	3.10	2.40	2.75	100
0.78					-	-	-	-
1.56	-	-	-	-	-	-	-	-
3.125	-	-	-	-	3.60	3.60	3.60	131
6.25	4.90	7.80	6.35	105	1.15	2.25	1.70	62
12.5	6.70	6.50	6.60	109	0.85	0.55	0.70	25
25	8.30	4.30	6.30	104	-	-	-	-
50	NM	NM	-	-				
EMS 500	3.40	4.30	3.85	64				
CP 25	-	-	-	-	1.40	2.45	1.93	70

- = not assessed NM = no scorable metaphases

Experiment 2: Mitotic Index (20-hour harvest)

Dose	Without S9	With S9

Level µg/ml	A	B	Mean	% of Control	A	B	Mean	% of Control
0	8.55	7.90	8.23	100	3.00	3.25	3.13	100
0.39					-	-	-	-
0.78					1.80	3.35	2.58	82
1.56					2.50	2.80	2.65	85
3.125	-	-	-	-	1.35	1.90	1.63	52
6.25	7.20	6.75	6.98	85	0.45	0.45	0.45	14
9.38					NM	NM	-	-
12.5	7.75	9.45	8.60	104				
25	6.00	9.45	7.73	94				
37.5	3.25	3.65	3.45	42				
50	NM	NM	NM	-				
EMS 500	4.70	7.95	4.83	59	-	-	-	-
CP 25	-	-	-	-	1.60	1.45	1.53	49

- = not assessed NM = no scorable metaphases

CONCLUSIONS

Remarks: The substance was found to be non-clastogenic under the conditions of the test.

DATA QUALITY

- **Reliabilities**: 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: Chromosome Aberration Test in Human Lymphocytes In Vitro, Report No. 814/002, 1995

Scott, D., Et al, Metaphase chromosome aberration assays in vitro. In: Kirkland, D.J., Basic mutagenicity tests: UKEMS recommended procedures. Report. Part 1 revised. Cambridge University Press, 1990:62-84

OTHER

- **Last Changed:** 25 April 2002
- **Order number for sorting:** 2

Remarks:

HUMAN HEALTH ENDPOINTS
15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)
Source: Cardolite Corporation, Lot No.: LP-2

METHOD

- **Method:** OECD 471
- **Test Type:** Reverse Mutation Assay (Ames Test)
- **System of testing:** Bacterial
- **GLP:** Yes
- **Year:** 1995
- **Species/Strain:** Salmonella typhimurium (TA1535, TA1537, TA1538, TA98 & TA100)
- **Metabolic activation:** S9-mix, Rat liver cells, 0.5 ml, Aroclor induced
- **Concentrations tested:** 50, 150, 500, 1500, 5000 µg/plate (±S9)
- **Statistical Methods:** Dunnett's method of linear regression

Remarks:

- **Test Design**

  - **Number of replicates:** 3
  - **Positive controls:** N-ethyl-N'-nitro-N-nitrosoguanidine (-S9, TA100 & TA1535)
    9-aminoacridine (-S9, TA 1537)
    4-nitro-o-phenylenediamine (-S9, TA1538)
    4-nitroquinoline-1-oxide (-S9, TA98)
    2-aminoanthracene (+S9, TA98, TA100, TA1535, TA1537 & TA1538)
  - **Negative control:** Solvent vehicle

- **Solvent:** Acetone

RESULTS

- **Result:** Negative
- **Cytotoxic concentration**

  - **With metabolic activation:** >5000 µg/plate
  - **Without metabolic activation:** >5000 µg/plate

- **Genotoxic effects**
  - **With metabolic activation:** None
  - **Without metabolic activation:** None

- **Statistical results:** No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material, either with or without metabolic activation.

Experiment 1 – Without Metabolic Activation

Test Substance Concentration (µg/plate)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	115 117 (110) 97 11.0	28 25 (21) 9 10.2	34 25 (25) 17 8.5	36 30 (30) 25 5.5	10 17 (15) 18 4.4
50	149 118 (131) 127 15.9	20 19 (19) 18 1.0	17 27 (23) 24 5.1	17 24 (21) 23 3.8	19 18 (18) 18 0.6
150	118 120 (120) 121 1.5	12 10 (12) 15 2.5	9 28 (16) 11 10.4	24 17 (18) 14 5.1	15 18 (17) 18 1.7
500	121 115 (116) 111 5.0	17 20 (16) 12 4.0	13 30 (18) 12 10.1	17 33 (25) 25 8.0	10 12 (12) 14 2.0
1500	115p 107p (117) 128p 10.6	8p 22p (16) 17p 7.1	15p 17p (15) 14p 1.5	22p 25p (27) 34p 6.2	13p 10p (12) 13p 1.7
5000	122p 85p (106) 111p 19.0	7p 15p (10) 8p 4.4	25p 15p (19) 18p 5.1	29p 22p (23) 17p 6.0	12p 19p (16) 18p 3.8
Positive Control	ENNG	ENNG	4NOPD	4NQO	9AA
Concentration (µg/plate)	3	5	5	0.2	80
Number of colonies per plate	670 933 (729) 583 182.2	198 213 (224) 260 32.3	470 474 (479) 494 12.9	168 152 (156) 149 10.2	76 208 (152) 172 68.2

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive controls: ENNG (N-ethyl-N'-nitro-N-nitroguanidine), 4NOPD (4-nitro-o-phenylenediamine), 4NQO (4-nitroquinloine-1-oxide), 9AA (9-aminoacridine)

Experiment 1 – With Metabolic Activation					
Test Substance Concentration (µg/plate)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537

0	131 108 (113) 101 15.7	19 17 (17) 15 2.0	39 35 (33) 24 7.8	24 38 (32) 35 7.4	17 18 (16) 14 2.1
50	118 129 (125) 127 5.9	18 13 (15) 15 2.5	34 24 (32) 39 7.6	29 34 (33) 35 3.2	17 13 (16) 17 2.3
150	121 111 (117) 120 5.5	13 19 (16) 17 3.1	32 33 (33) 33 0.6	33 33 (35) 39 3.5	10 15 (15) 19 4.5
500	111 143 (115) 91 26.2	10 15 (15) 19 4.5	18 35 (29) 34 9.5	35 38 (33) 27 5.7	12 15 (12) 10 2.5
1500	98p 133p (114) 112p 17.6	20p 17p (19) 20p 1.7	25p 28p (27) 28p 1.7	28p 28p (30) 33p 2.9	13p 18p (15) 13p 2.9
5000	128p 112p (117) 112p 9.2	12p 17p (14) 13p 2.6	30p 18p (24) 25p 6.0	29p 28p (29) 30p 1.0	14p 19p (17) 17p 2.5
Positive Control	2AA	2AA	2AA	2AA	2AA
Concentration (µg/plate)	1	2	0.5	0.5	2
Number of colonies per plate	1332 1615(1382) 1200 212.0	64 69 (66) 64 2.9	353 323 (361) 408 43.1	219 453 (329) 315 117.6	194 252 (232) 250 32.9

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive control: 2AA (2-aminoanthracene)

Experiment 2 – Without Metabolic Activation					
Test Substance Concentration (µg/plate)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	107 132 (116) 110 13.7	14 18 (17) 19 2.6	29 22 (21) 12 8.5	17 23 (21) 24 3.8	19 19 (17) 13 3.5

50	149 133 (140) 138 8.2	12 18 (21) 32 10.3	41 22 (29) 23 10.7	34 30 (32) 0	10 20 (14) 13 5.1
150	133 118 (129) 137 10.0	18 24 (24) 29 5.5	10 22 (17) 19 6.2	35 33 (32) 28 3.6	14 10 (12) 12 2.0
500	134 139 (131) 121 9.3	13 13 (15) 20 4.0	20 14 (21) 30 8.1	23 34 (25) 19 7.8	13 19 (19) 24 5.5
1500	117p 117p (109) 92p 14.4	12p 10p (12) 14p 2.0	22p 23p (21) 18p	17p 20p (26) 40p 12.5	18p 12p (15) 14p 3.1
5000	107p 121p (108) 95p 13.0	10p 25p (19) 23p 8.1	19p 19p (20) 23p 2.3	20p 24p (28) 39p 10.0	14p 12p (12) 10p 2.0
Positive Control	ENNG	ENNG	4NOPD	4NQO	9AA
Concentration (µg/plate)	3	5	5	0.2	80
Number of colonies per plate	916 711 (711) 740 110.9	514 504 (498) 477 19.1	406 499 (455) 459 46.7	177 203 (196) 208 16.6	638 656 (589) 474 100.3

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive controls: ENNG (N-ethyl-N'-nitro-N-nitroguanidine), 4NOPD (4-nitro-o-phenylenediamine), 4NQO (4-nitroquinloine-1-oxide), 9AA (9-aminoacridine)

Experiment 2 – With Metabolic Activation					
Test Substance Concentration (µg/plate)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	137 139 (131) 117 12.2	25 20 (20) 15 5.0	35 28 (34) 39 5.6	24 28 (32) 44 10.6	22 18 (18) 13 4.5
50	133 138 (128) 112 13.8	24 22 (21) 18 3.1	31 33 (32) 32 1.0	38 33 (34) 32 3.2	19 24 (19) 13 5.5

150	108 120 (115) 118 6.4	23 30 (25) 22 4.4	25 35 (34) 43 9.0	29 36 (33) 35 3.8	14 22 (17) 14 4.6
500	122 142 (125) 110 16.2	23 24 (23) 23 0.6	23 30 (26) 25 3.6	28 24 (32) 44 10.6	13 13 (16) 22 5.2
1500 5000	129p 120p (123) 121p 4.9 128p 170p (135) 106p 32.5	13p 15p (19) 30p 9.3 18p 20p (18) 15p 2.5	25p 22p (25) 28p 3.0 32p 17p (29) 38p 10.8	28p 13p (26) 36p 11.7 36p 35p (36) 36p 0.6	17p 17p (16) 14p 1.7 15p 15p (15) 14p 0.6
Positive Control	2AA	2AA	2AA	2AA	2AA
Concentration (µg/plate)	1	2	0.5	0.5	2
Number of colonies per plate	1398 1553(140 6) 1268 142.7	102 139 (114) 102 21.4	276 256 (273) 286 15.3	159 293 (243) 278 73.4	255 250 (254) 258 4.0

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive Control: 2AA (2-aminoanthracene)

Remarks: A precipitate was observed at and above 1500 µg/plate, however this did not interfere with the scoring of revertant colonies.

CONCLUSIONS

Remarks: The substance was found to be non-mutagenic under the conditions of the test.

DATA QUALITY

- **Reliabilities**: 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: Reverse Mutation Assay 'Ames Test' Using Salmonella Typhimurium, Report No. 814/001, 1995

Kirkland, D.J., (Ed), Statistical Evaluation of Mutagenicity Test Data, UKEMS Sub-committee on Guidelines for Mutagenicity Testing, Report - Part III (1989), Cambridge University Press

OTHER

- **Last Changed:** 25 April 2002
- **Order number for sorting:** 1

Remarks:

ENVIRONMENTAL FATE ELEMENTS AND PATHWAYS
9. BIODEGRADATION

TEST SUBSTANCE

- **Identity:** Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)
Source: Cardolite Corporation. Lot No.: LP-2

METHOD

- **Method:** OECD Method 302D
- **Test Type:** aerobic
- **GLP:** Yes
- **Year:** 1993
- **Contact time:** 28 (days)
- **Innoculum:** activated sludge

Remarks:

- **Innoculum:** Fresh activated sludge from a municipal biological sewage treatment plant. 30 mg suspended solids/l of test medium.
- **Concentration of test chemical:** 6.01 – 6.39 mg, direct addition
- **Temperature of incubation:** 20°C
- **Dosing procedure:** Test substance weighed on a piece of glass to an amount of about 20 mg ThOD (or COD) and added directly to the test flask.
- **Sampling frequency:** 0,7,14,21 & 28 days
- **Controls:** Sodium acetate used as positive control, innoculum used as blank.
- **Analytical method used to measure biodegradation:** The COD of the poorly soluble substance was determined in a variation of ISO Method 6060 (closed system with a pressure equaliser / Kelkenberg method, Z.f. Wasser und Abwasserforschung (1975) 146). Oxygen determination was performed using an oxygen electrode (WTW;FRG; Microprocessor oximeter OXI 2000 with electrode model TriOxmatic EO 200).
- **Method of calculating measured concentrations:** Arithmetic mean

RESULTS

- **Degradation % after time:** 96% after 28 days
- **Results:** Readily biodegradable
- **Kinetic:**

Day	% Degradation	
	Sample	Positive control
7	46	75
14	72	86
21	86	91
28	96	97

- **Breakdown products:** Not determined

Remarks: None

CONCLUSIONS

Remarks:

According to the author of the study, based on the data (i.e. 96% degradation after 28 days) Cardolite NC-511 can be regarded as very highly biodegradable.

DATA QUALITY

- **Reliabilities:** 1, Reliable without restriction

Remarks: Study conducted under GLP to recognised test method by Henkel KGaA

REFERENCES

Henkel KGaA, Cardolite NC-511 Ultimate biodegradability in the BODIS-Test, Report No. RE930104, 1993

OTHER

Last Changed: 23 April 2002
Order number for sorting: 1

Remarks:

The test method used was based on the closed bottle test (OECD test method 302D) and the RDA-Blok test, previously published (Blok, J., A Repetitive Die Away (RDA) Test Combining Several Biodegradability Test Procedures, Int. Biodeterior. Bull., 15 (1979) 57-63) and ring-tested by the OECD (1988 ring test on ready biodegradability).

ECOTOXICITY ENDPOINTS
10. ACUTE TOXICITY TO FISH

TEST SUBSTANCE

- **Identity:** Cashew Nut Shell Liquid

Remarks: Test substance: Cardanol (CAS No. 37330-39-5), Cardol (CAS No. 57486-25-6)

METHOD

- **Method:** Calculation using ECOSAR v.0.99e
- **Type:** N/A
- **GLP:** No
- **Year:** 2002
- **Species:** Fish

Remarks: None

RESULTS

- **Unit:** mg/L
- **LC_{50} at 96 hours:** 0.002 – 0.005 (Cardanol)
  0.005 – 0.011 (Cardol)

Remarks: The predicted LC_{50} values vary with the degree of unsaturation in the alkyl side chain of Cardanol and Cardol as follows:

	LC_{50}, mg/L			
	unsaturated	monoene	diene	triene
Cardanol	0.002	0.003	0.004	0.005
Cardol	0.005	0.007	0.009	0.011

CONCLUSIONS

Remarks:

Estimation using ECOSAR v0.99e predict Cardanol and Cardol, the two major components of distilled and technical grade Cashew Nut Shell Liquid, to be toxic to fish.

DATA QUALITY

- **Reliabilities:** 4, Not Assignable

Remarks:

Estimation using ECOSAR v0.99e

REFERENCES

ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

OTHER

- Last Changed: 24 April 2002
- Order number for sorting: 1

Remarks:

ECOTOXICITY ENDPOINTS
12. TOXICITY TO AQUATIC INVERTIBRATES (E.G., DAPHNIA)

TEST SUBSTANCE

- **Identity:** Cashew Nut Shell Liquid

Remarks: Test substance: Cardanol (CAS No. 37330-39-5), Cardol (CAS No. 57486-25-6)

METHOD

- **Method:** Calculation using ECOSAR v.0.99e
- **Type:** N/A
- **GLP:** No
- **Year:** 2002
- **Species:** Daphnia

Remarks: None

RESULTS

- **Unit:** mg/L
- **LC_{50} at 48 hours:** 0.024 – 0.040 (Cardanol)
  0.039 – 0.066 (Cardol)

Remarks: The predicted LC_{50} values vary with the degree of unsaturation in the alkyl side chain of cardanol and cardol as follows:

	LC_{50}, mg/L			
	unsaturated	monoene	diene	triene
Cardanol	0.024	0.029	0.035	0.040
Cardol	0.039	0.048	0.058	0.066

CONCLUSIONS

Remarks:

Estimation using ECOSAR v0.99e predicts Cardanol and Cardol, the two major components of distilled and technical grade Cashew Nut Shell Liquid, to be toxic to Daphnia.

DATA QUALITY

- **Reliabilities:** 4, Not Assignable

Remarks:

Estimation using ECOSAR v.0.99e

REFERENCES

ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

OTHER

- Last Changed: 24 April 2002
- Order number for sorting: 1

Remarks:

HUMAN HEALTH ENDPOINTS
15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)
Source: Cardolite Corporation, Lot No.: LP-2

METHOD

- **Method:** OECD 476
- **Test Type:** Forward Mutation Assay
- **System of testing:** Non bacterial
- **GLP:** Yes
- **Year:** 1996
- **Species/Strain:** Chinese Hamster Ovary CHO-KI BH4
- **Metabolic activation:** S9-mix, Rat liver cells, Aroclor induced
- **Concentrations tested:** Expt. 1: 0, 0.75, 1.5, 3, 6, 12 µg/ml (-S9)
  0, 1.5, 3, 6, 12, 18 µg/ml (+S9)
  Expt. 2: 0, 0.75, 1.5, 3, 6, 9 µg/ml (-S9)
  0, 3, 6, 12, 18, 24 µg/ml (+S9)

- **Statistical Methods:** Cochran-Armitage test for trend analysis, Fisher-Irwin exact test for group comparisons for proportions.

Remarks:

- **Test Design**

  - **Number of replicates:** 2
  - **Positive control:** Ethyl methanesulphonate (EMS) (-S9), 3-methylcholanthrene (3-MC) (+S9)
  - **Negative control:** Solvent vehicle

- **Solvent:** Dimethylsulfoxide

RESULTS

- **Result:** Negative
- **Cytotoxic concentration**

  - **With metabolic activation:** 47.19 µg/ml
  - **Without metabolic activation:** 47.19 µg/ml

- **Genotoxic effects**
  - **With metabolic activation:** None
  - **Without metabolic activation:** None

- **Statistical results:** The test material did not induce significant or dose-related increases in mutant frequency per survivor in either the presence or absence of metabolic activation in either of the two experiments.

Summary of Results:

Experiment 1:

Dose Level µg/ml	Without S9		Mean MFS	Dose Level µg/ml	With S9		Mean MFS
	A	B			A	B	
0	3.4	0.7	2.05	0	3.5	3.3	3.4
0.75	1.4	-	1.4	1.5	2.9	0.7	1.80
1.5	2.0	0.0	1.00	3.0	0.6	1.4	1.00
3	0.0	0.0	0.0	6.0	2.9	0.0	1.45
6	0.0	0.0	0.0	12	1.4	6.3	3.85
12	0.0	6.3	3.15	18	0.7	8.6	4.65
EMS 200	154.5	189.9	172.20	24	-	-	-
				3-MC 4	238.8	285.9	262.35

MFS = 6-TG resistant mutants/10^6 viable cells

Experiment 2:

Dose Level µg/ml	Without S9		Mean MFS	Dose Level µg/ml	With S9		Mean MFS
	A	B			A	B	
0	0.0	0.6	0.30	0	8.1	0.8	4.45
0.75	0.4	7.6	4.00	3	1.3	0.0	0.65
1.5	3.2	0.9	2.05	6	0.9	0.0	0.45
3	0.6	6.2	3.40	2	0.0	0.0	0.00
6	0.5	1.7	1.10	18	0.0	0.0	0.00
9	0.6	0.0	0.30	24	TOXIC		
EMS 200	158.3	149.1	153.70				
				3-MC 4	284.6	278.1	281.35

MFS = 6-TG resistant mutants/10^6 viable cells

Remarks:

CONCLUSIONS

Remarks: The test material was found to be non-mutagenic to CHO cells at the HGPRT locus under the conditions of this test.

DATA QUALITY

- **Reliabilities**: 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: CHO HGPRT Forward Mutation Assay, Report No. 814/003, 1996

Cole, J., et al, (1990): Gene Mutation in Cultured Mammalian Cells. In 'Basic Mutagenicity Tests: UKEMS Recommended Procedures', (ed D.J. Kirkland), Cambridge University Press, New York,

OTHER

- **Last Changed:** 26 April 2002
- **Order number for sorting:** 3

Remarks: